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U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 100, PART VII.

B. T. GALLOWAY, *Chief of Bureau.*

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THE EFFECT OF COPPER UPON WATER BACTERIA.

BY

KARL F. KELLERMAN,
PHYSIOLOGIST IN CHARGE OF SOIL BACTERIOLOGY AND WATER PURIFICATION INVESTIGATIONS,

AND

T. D. BECKWITH,
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THE EFFECT OF COPPER UPON WATER BACTERIA.[a]

INTRODUCTION.

Of the many methods of chemical treatment for water purification, the copper method has recently received the most attention. The use of this metal, or its salts, was urged primarily[b] for the eradication or control of polluting algæ, tho popular attention has been directed more particularly to the various results obtained in destroying the typhoid bacillus.

The discrepancies between laboratory results of some of the investigators of this phase of the subject have deterred many engineers and sanitarians from conducting important tests of the value of copper under emergency or epidemic conditions. These discrepancies are perhaps in some cases due to reasons which are taken up later in this bulletin, and in some cases to the lack of comprehension of the essential difference between treatment of drinking water which contains relatively little albuminoid matter[c] and treatment of bouillons or emulsions.[d]

[a] The copper method for controlling algal pollution can no longer be considered in an experimental stage. The accumulation of results of treatments constantly made under the direct supervision of officials of the Department of Agriculture and the numerous results reported to this Department by independent experimenters render further discussion of this question superfluous. The caution should be reiterated, however, that no rule for determining the amount of copper sulfate to be added can be given, and each body of water must be treated in the light of its special conditions. This caution is eminently applicable to copper treatment of a water supply for bactericidal purposes, and, as Mr. Kellerman and Mr. Beckwith have shown in their recent work, the problem of using dilute solutions of copper for destroying *Bacillus coli* and *Bacillus typhi* in water is a rather complicated one. Pertinent conditions must be understood thoroly before an application of copper can be safely advised, and whether an emergency treatment of an unfiltered and contaminated water, or a continuous treatment of a filtered water supply, or a treatment of a sewage effluent is contemplated the work should be supervised by an expert.—A. F. Woods, *Pathologist and Physiologist, Acting Chief of Bureau.*

[b] Buls. 64 and 76, Bureau of Plant Industry, U. S. Dept. of Agriculture.

[c] See also Buls. 64 and 76, Bureau of Plant Industry, U. S. Dept. of Agriculture.

[d] A case in point is a paper by Dr. J. B. Thomas, Report on the Action of Various Substances on Pure Cultures of the Amœba Dysenteriæ. (Amer. Jour. Med. Sci., vol. 131, No. 1, Jan., 1906, p. 116.) "Uniform suspensions of the amœba were made by pouring 4 cc. of distilled sterile water over the surface of a 48-hour slant agar culture of the amœba and cholera spirillum, scraping off the surface growth and mixing with the matter by means of a platinum wire, and pouring the resultant emulsion into a sterile test tube; 4 cc. of the antiseptic solution (in double strength)

In spite of the fact that the field results of several experimenters[a] have apparently established the point that copper sulfate will not destroy ordinary water bacteria at concentrations fatal to the colon and typhoid bacilli, the somewhat fanciful objection has been suggested to chemical treatment of any kind, and to copper treatment in particular, that bacteria desirable for oxidation of organic matter and other beneficial changes in a water supply might be injured equally with the typhoid bacteria, and thus a treated water be more potentially dangerous than an untreated water known to contain typhoid organisms; in other words, that many bacteria which could be of decided benefit in a slightly polluted water supply might be eradicated by copper treatment and that in this way the fractional sterilization of a reservoir by copper might pave the way for a more dangerous contamination.

Investigations of these and other points have been undertaken, and it is believed that valuable data have been obtained, applicable to copper treatment for algæ, to emergency treatment for typhoid, and to copper treatment in connection with filtration. The copper treatment of sewage may be influenced by the same conditions that bear upon the effect of copper treatment of water. For the present, however, the investigations of Johnson,[b] at the Columbus, Ohio, sewage testing station may be considered sufficiently accurate for practical purposes.

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to be tested was then added to the 4 cc. emulsion of amœbæ, thus making a fairly uniform emulsion of 8 cc. of liquid to one 48-hour slant culture, the mixture containing a definite amount of the chemical to be tested. * * * It would appear from the above results that it would be disastrous to rely on the action of copper containers to purify water infected with amœbæ or cholera."

[a] Caird. Copper Sulphate Results. Paper read at meeting of American Water Works Association, Boston, Mass., July 10–14, 1906.

Jackson. Journal New England Water Works Association, vol. 19, 1905, pp. 563–568.

Hollis. Journal New England Water Works Association, vol. 19, 1905, pp. 571–572.

Stokes and Thomas. The Effect of Copper Sulphate upon the Bacteriological and Chemical Constituents of Large Bodies of Water. Public Health Papers and Reports, American Public Health Association, vol. 31, part 1, 1905, pp. 75–90.

[b] The Copper Treatment of Sewage Effluents. Report on Sewage Purification at Columbus, Ohio, 1905.

"Available data indicate that the removal by either process of applied pathogenic and nonpathogenic bacteria is in fairly direct proportion, generally speaking, although, of course, saprophytic bacteria may multiply within the tanks or filters, so as to obscure the true removal. Under some conditions it might be advantageous to employ a germicide, such as sulphate of copper, as a final treatment for sewage effluents of doubtful bacterial purity." (P. 471.)

"Independent of the question of complete sterilization as touched upon * * * it may be that there is a field of usefulness in some places for copper sulphate or other germicidal chemical in the treatment of coarse-grain filter effluents, in order to bring them from a bacterial or hygienic standpoint to a degree of purity strictly comparable with that of the effluent of ordinary intermittent sand filters." (P. 479.)

RESISTANCE OF VARIOUS BACTERIA.

Several species of bacteria occurring commonly in Potomac River water have been isolated, and comparative tests have been carried on with these bacteria and with the colon bacillus. An examination of the tables showing the exact results of these tests indicates clearly that no danger to most of the common saprophytic bacteria can be expected from using concentrations of copper sufficiently strong to destroy *Bacillus coli*. To allow as little variation as possible in the different tests all experiments were carried out in triple-distilled water. This will probably explain the sensitiveness of most of the bacteria to copper solutions, for, as Gildersleeve[a] has stated, "The resistance of bacteria washed in distilled water is lessened, due in all probability to the fact that some of the slimy substance which surrounds the bacterial cell and protects it to some extent from the action of detrimental agencies is removed." For purposes of comparison the table showing the action of copper sulfate on various bacteria in tap water, published by Gildersleeve,[b] is reproduced below.

Organism.	Dilution.	Average number to the cubic centimeter in control.	Percentage destroyed in hours specified.												
			1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	24.
B. typhosus	1: 250,000	110,000	100	....	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	100,000	99	100	....	....	....	....	....	....	....	....	....	....	....
	1:1,000,000	140,000	78	100	....	....	....	....	....	....	....	....	....	....	....
	1:1,500,000	120,000	50	85	99	100	....	....	....	....	....	....	....	....	....
	1:2,000,000	130,000	4	7	55	80	82	87	87	93	98	100	....	....	....
B. coli communis	1: 250,000	105,000	100	....	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	120,000	99	100	....	....	....	....	....	....	....	....	....	....	....
	1:1,000,000	99,000	80	99	100	....	....	....	....	....	....	....	....	....	....
	1:1,500,000	150,000	87	95	99	100	....	....	....	....	....	....	....	....	....
	1:2,000,000	110,000	8	10	11	27	50	85	90	92	93	93	95	98	100
B. dysenteriæ	1: 250,000	11,530	95	100	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	120,000	40	95	97	100	....	....	....	....	....	....	....	....	....
	1:1,000,000	72,000	31	50	62	85	99	99+	100	....	....	....	....	....	....
	1:1,500,000	12,400	20	30	40	42	60	70	75	81	85	87	90	91	100
	1:2,000,000	13,600	20	20	25	30	30	45	60	62	62	68	71	75	98
B. cloacæ	1: 250,000	92,000	90	99	100	....	....	....	....	....	....	....	....	....	....
	1: 500,000	86,000	68	90	99	100	....	....	....	....	....	....	....	....	....
	1:1,000,000	101,000	33	85	89	96	96	99	100	....	....	....	....	....	....
	1:1,500,000	89,000	5	10	30	40	70	85	95	98	98	99	100	....	....
	1:2,000,000	94,500	3	10	31	40	80	83	85	88	91	91	93	97	100
B. proteus vulgaris	1: 250,000	115,000	93	100	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	106,000	65	92	98	100	....	....	....	....	....	....	....	....	....
	1:1,000,000	111,000	28	51	82	90	95	98	100	....	....	....	....	....	....
	1:1,500,000	99,000	10	18	27	42	69	90	93	98	99+	100	....	....	....
	1:2,000,000	118,000	0	12	22	36	51	65	78	80	85	88	92	95	100
B. prodigiosus	1: 250,000	102,000	95	99	99	99+	100	....	....	....	....	....	....	....	....
	1: 500,000	109,900	15	26	80	95	97	99	99+	100	....	....	....	....	....
	1:1,000,000	110,000	9	11	16	33	33	45	47	61	63	69	....	....	93
	1:1,500,000	107,000	4	12	17	30	38	46	46	48	51	52	60	61	92
	1:2,000,000	98,000	0	1	8	8	10	11	15	30	38	41	43	50	88
Staphylococcus pyogenes aureus	1: 250,000	92,000	99	100	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	88,000	50	70	90	100	....	....	....	....	....	....	....	....	....
	1:1,000,000	86,000	15	30	30	70	78	85	97	99	99	100	....	....	....
	1:1,500,000	100,000	8	22	28	96	58	69	85	93	95	99	100	....	....
	1:2,000,000	95,000	1	8	10	30	50	64	79	82	85	98	99	99+	100

[a] Studies on the Bactericidal Action of Copper on Organisms in Water. American Journal of Medical Science, vol. 129, 1905, p. 754.

[b] Op. cit., p. 759.

The figures in this table are rather surprizing in connection with the statement that "very little difference was found between distilled and filtered water used in the laboratory." The similarity here of the action of copper in distilled and filtered tap water, contrasted with the marked difference in the action of distilled and filtered tap water in Washington, emphasizes a point previously mentioned,[a] that the water itself deserves as careful a study as do the organisms contained therein. This is suggested again in Phelps's[b] report showing that with either hard or turbid waters the germicidal efficiency of metallic copper is much lessened. Again, in view of the high toxicity that investigators have reported with copper in Philadelphia tap water, it seems necessary to assume that this water either is peculiarly favorable to maintaining the metal in a toxic state, or, what seems equally probable, for rendering the bacteria unusually sensitive.

The results of our own experiments are given in the following tables. All the experiments, unless otherwise indicated, were conducted in Weber resistance glass test tubes, each containing 10 c. c. of water triple distilled from glass, portions of which had been treated previously with the desired amount of copper sulfate. All tubes were inoculated with a 2 mm. loop of the proper organism. The temperature during each experiment varied from 18° to 22° C.

TABLE I.—*Effect of copper sulfate upon Bacillus mycoides.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	105	90	590	310	70	250	9
6 hours	80	1	20	80	15	35	1
24 hours	185	10	130	110	40	20	5

TABLE II.—*Effect of copper sulfate upon Bacillus megatherium.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	70	55	45	30	90	15	5
2 hours	50	10	25	30	40	0	2
6 hours	40	15	20	10	40	1	1
24 hours	200	10	15	5	10	2	1

[a] Bul. 76, Bureau of Plant Industry, U. S. Dept. of Agriculture, p. 12; and Kellerman, Journal New England Water Works Association, vol. 19, 1905, p. 536.

[b] Journal New England Water Works Association, vol. 19, 1905, pp. 537–539.

TABLE III.—*Effect of copper sulfate upon Bacillus mesentericus.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	300	380	400	520	280	440	470
2 hours	280	170	310	440	260	340	360
6 hours	430	10	290	320	250	310	390
24 hours	1,350	0	0	530	3	4	30

TABLE IV.—*Effect of copper sulfate upon Bacillus mesentericus fuscus.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	30	40	25	25	50	20	30
2 hours	0	5	50	10	3	0	1
6 hours	2	1	30	5	5	2	1
24 hours	70	110	70	45	0	20	80

TABLE V.—*Effect of copper sulfate upon Streptococcus pyogenes.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	180	875	875	300	250	90	180
2 hours	210	0	0	290	180	180	180
6 hours	140	0	0	280	230	240	160
24 hours	35	0	0	390	150	10	20

TABLE VI.—*Effect of copper sulfate upon Bacillus subtilis.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	310	80	20	130	85	100	30
2 hours	220	1	3	30	120	70	10
6 hours	330	1	1	25	85	50	2
24 hours	2,800	1	0	30	70	65	0

TABLE VII.—*Effect of copper sulfate upon Bacillus prodigiosus.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	350	450	400	1,050	22,000	2,000	1,800
2 hours	750	65	180	1,700	1,350	2,050	1,550
6 hours	1,250	1	0	2	0	550	200
24 hours	925	0	0	0	0	0	0

TABLE VIII.—*Effect of copper sulfate upon Bacillus liquifaciens phosphorescens.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	16,500	3,400	2,400	16,000	4,500	10,400	13,000
2 hours	15,800	0	1	0	5	2,900	7,300
6 hours	23,000	0	0	0	0	640	7,000
24 hours	36,000	0	0	0	0	60	5,300

TABLE IX.—*Effect of copper sulfate upon pink yeast.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	310	550	100	110	100	220	108
2 hours	150	5	1	0	1	65	85
6 hours	70	0	0	0	1	3	50
24 hours	90	0	0	0	0	1	30

TABLE X.—*Effect of copper sulfate upon Bacillus coli.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.	2,000,000 parts of water.	3,000,000 parts of water.	4,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	2,650	2,300	3,100	2,700	2,250	1,800	1,770
2 hours	2,350	1,650	2,650	2,000	1,650	1,850	1,850
6 hours	2,300	0	0	95	210	850	1,070
24 hours	6,300	0	0	0	0	0	65

TABLE XI.—*Effect of copper sulfate upon sulfur yellow bacillus.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	2,800	40	3,100	2,800	4,100	860	1,300
2 hours	2,000	0	0	0	280	1	2
6 hours	1,500	0	0	0	0	0	0
24 hours	24,000	0	0	0	0	0	0

TABLE XII.—*Effect of copper sulfate upon Pseudomonas radicicola (soy).*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	1,900	520	1,300	2,800	650	520	3,100
2 hours	1,800	0	0	45	0	25	1,100
6 hours	2,100	0	0	0	0	0	30
24 hours	18,000	0	0	0	0	0	0

TABLE XIII.—*Effect of copper sulfate upon Bacillus sublanatus.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	12,500	780	2,700	2,100	1,100	2,100	1,500
2 hours	12,000	0	0	0	0	35	190
6 hours	12,500	0	0	0	0	0	80
24 hours	40,000	0	0	0	1	0	0

TABLE XIV.—*Effect of copper sulfate upon Micrococcus radians.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	1,200	2,700	10,500	1,700	3,800	2,500	2,800
2 hours	1,200	1	0	1	20	85	10
6 hours	1,400	0	0	0	0	0	1
24 hours	1,900	0	0	0	0	0	0

TABLE XV.—*Effect of copper sulfate upon Pseudomonas radicicola (alfalfa).*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	270	300	330	425	3,700	700	290
2 hours	210	0	0	0	0	0	0
6 hours	220	0	0	0	0	0	0
24 hours	1,175	0	0	0	0	0	0

TABLE XVI.—*Effect of copper sulfate upon Bacillus violaceus laurentius.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	7,200	120	480	740	470	210	520
2 hours	5,300	0	0	0	0	1	25
6 hours	6,800	0	0	0	0	0	0
24 hours	40,000	0	0	0	0	0	0

TABLE XVII.—*Effect of copper sulfate upon Pseudomonas amethystina.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	140	540	90	70	580	670	660
2 hours	190	0	0	1	3	3	15
6 hours	240	0	0	0	0	1	1
24 hours		0	0	0	0	0	0

TABLE XVIII.—*Effect of copper sulfate upon Bacillus caudatus.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	875	60	680	2,100	15	70
2 hours	350	0	0	1	0	0
6 hours	400	0	0	1	0	0
24 hours	9,000	0	0	0	0	0

TABLE XIX.—*Effect of copper sulfate upon Bacillus rubrum.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	175	10	30	225	90	210	135
2 hours	195	0	0	90	0	0	0
6 hours	50	0	0	5	0	0	0
24 hours	1,050	0	0	5	0	0	0

EFFECT OF CARBON DIOXID ON VIABILITY OF BACILLUS COLI AND BACILLUS TYPHI.

A careful study of the gas content of both tap and triple distilled water has shown that for the typhoid and colon bacilli the presence of carbon dioxid in the water is associated with heightened resistance to toxic agents, such as solutions of copper salts, precipitated copper salts, and copper metal. This is the more strange when one considers that the presence of carbon dioxid in water causes the copper to remain in solution, and in case of insoluble copper, either precipitated or metallic, the carbon dioxid serves to bring a considerable amount of copper into solution. It should be noted, however, that water heavily saturated with carbon dioxid is toxic to *Bacillus coli* and *Bacillus typhi*.

A series of experiments designed to test the effect of carbon dioxid on *Bacillus coli*, the various conditions of triple distilled water with and without copper, triple distilled water plus calcium carbonate with and without copper, and tap water with and without copper are tabulated below.

TABLE XX.—*Toxicity of copper sulfate to Bacillus coli in the absence of carbon dioxid.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	6,800	1,400	2,300	2,200	900	3,500
2 hours	5,100	0	0	0	2	80

[1] Experiment conducted in Weber resistance glass test tubes each containing 10 c. c. of water triple distilled from glass, portions of which had been treated previously with the desired amount of copper sulfate. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

TABLE XXI.—*Effect of carbon dioxid upon toxicity of copper sulfate to Bacillus coli.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	9,200	8,200	5,800	11,600	7,560	6,200
2 hours	2,000	0	110	38	900	350

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of water triple distilled from glass, portions of which had been treated previously with the desired amount of copper sulfate. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

TABLE XXII.—*Effect of water containing calcium monocarbonate and various quantities of copper sulfate upon Bacillus coli.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies*	*Colonies.*
0 hour	2,950	3,510	2,670	3,150	4,290	3,760
6 hours	240	0	0	0	105	80
24 hours	5	0	0	0	0	0

[1] Various dilutions of copper sulfate were tubed in Weber resistance glass, thoroly boiled, a small measured quantity of calcium carbonate added to each tube, and these solutions cooled and kept in a Novy jar in an atmosphere free of carbon dioxid. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from the Bureau of Animal Industry and isolated from hog. The temperature during this experiment varied from 18° to 22° C.

TABLE XXIII.—*Effect of carbon dioxid content of water containing calcium carbonate and various quantities of copper sulfate upon Bacillus coli.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	4,650	4,730	3,850	3,980	4,100	4,470
6 hours	4,500	0	0	5	415	620
24 hours	5,200	0	0	0	15	60

[1] Various dilutions of copper sulfate were tubed in Weber resistance glass, thoroly boiled, a small measured quantity of calcium carbonate added to each tube, and these solutions cooled and kept in a Novy jar in an atmosphere composed largely of carbon dioxid. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from the Bureau of Animal Industry and isolated from hog. The temperature during this experiment varied from 18° to 22° C.

TABLE XXIV.—*Toxicity of copper sulfate to Bacillus coli in tap water free of carbon dioxid.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	6,300	7,600	6,000	1,050	4,800	5,200
2 hours	1,500	0	0	0	Lost.	60
6 hours	280	0	0	0	0	0
24 hours	0	0	0	0	0	0

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of Potomac tap water, portions of which had been treated previously with the desired amount of copper sulfate. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from the Bureau of Animal Industry, isolated from hog. The temperature during this experiment varied from 18° to 22° C.

TABLE XXV.—*Toxicity of copper sulfate to Bacillus coli in tap water free of carbon dioxid.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	4,600	1,800	4,800	3,700	4,500	4,000
2 hours	1,450	0	0	20	80	100
6 hours	1,500	0	0	0	5	2
24 hours	250	0	0	0	0	0

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of Potomac tap water, portions of which had been treated previously with the desired amount of copper sulfate. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

TABLE XXVI.—*Effect of carbon dioxid upon toxicity of copper sulfate to Bacillus coli.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	4,200	4,000	9,800	1,270	5,100	1,100
2 hours	2,800	10	0	0	110	260
6 hours	1,460	15	15	20	10	35
24 hours	1,540	0	0	0	0	0

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of Potomac tap water, portions of which had been treated previously with the desired amount of copper sulfate. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from the Bureau of Animal Industry, isolated from hog. The temperature during this experiment varied from 18° to 22° C.

TABLE XXVII.—*Effect of carbon dioxid upon toxicity of copper sulfate to Bacillus coli.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	3,800	3,000	8,500	5,200	4,400	5,500
2 hours	2,300	0	5	15	2,150	1,500
6 hours	1,600	10	12	10	120	100
24 hours	3,500	0	0	10	35	0

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of Potomac tap water, portions of which had been treated previously with the desired amount of copper sulfate. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

An examination of the foregoing tables shows that with the three types of water the presence of carbon dioxid increases the resistance of the bacilli in question. In the solution containing monocarbonate of lime and copper sulfate the bacteria are extremely sensitive, even the bacteria in the check solutions dying rapidly, while in the solutions charged with carbon dioxid the bacteria were able to persist in considerable numbers in the dilute copper solutions in spite of the fact that most of the copper must have remained in solution. This point is interesting in connection with the work of Engels,[a] who reports a

[a] Weitere Studien über die Sterilization von Trinkwasser auf chemischen Wege. Centralbl. f. Bakt., Parasit., u. Infekt., vol. 32 Orig., 1902, pp. 495–521.

rather high toxicity for calcium chlorid, and of Pfuhl,[a] who suggests a calcium salt—milk of lime—for water sterilization. The use of lime or a similar agent may be highly desirable in connection with treating a contaminated reservoir with copper, tho the inferior germicidal power of lime makes it improbable that the latter alone could be used safely.

In regard to chemical water analysis, it seems probable that the determination of carbon dioxid or the determination of monocarbonate and bicarbonate alkalinity may have importance hitherto unrecognized, and the variations in the longevity determinations of *Bacillus coli* and *Bacillus typhi* may be due in part to the mineral constituents of a water, in part to methods of experimentation, and in part to the carbon dioxid content. Extended field tests must be made before generalizations on the possible effect of the gas content of a water supply can be determined.

The carbon dioxid content of a water may possibly explain the peculiar results obtained by Clark and Gage.[b] They have reported practically no toxic action from metallic copper, or at least very little difference in the action of metallic copper, iron, tin, zinc, and lead. Their figures are rather misleading because of the great number of days the experiments were carried on, and, as Phelps[c] has shown, metallic copper is coated with some insoluble substance after a few days' exposure to Boston tap water and no longer has great toxic action. The lack of toxicity of metallic copper and the similarity of its action to the action of other metals, as reported by Clark and Gage, is entirely at variance with the work of Kraemer,[d] Pennington,[e] Gildersleeve,[f] Stewart,[g] and Moore and Kellerman.[h] Investigators generally have agreed that it would be possible to practically sterilize

[a] Über die Disinfection der Typhus and Cholera-ausleerungen mit Kalk. Zeitschr. f. Hyg. u. Infekt., vol. 6–s, 1889, pp. 97–104.

[b] The Use of Copper Sulphate in Water Filtration. Journal of Infectious Diseases. Supplement No. 2, February, 1906, pp. 172–174.

[c] Experiments on the Storage of Typhoid Infected Water in Copper Canteens. Public Health Papers and Reports, American Public Health Association, vol. 31, part 1, 1905, pp. 75–90.

[d] Copper Treatment of Water. American Journal of Pharmacy, vol. 76, December, 1904, pp. 574–579.

The Use of Copper in Destroying Typhoid Organisms and the Effects of Copper on Man. American Journal of Pharmacy, vol. 77, June, 1905, pp. 265–181.

[e] The Action of Electrically Charged Copper Upon Certain Organisms in Water. American Journal of Medical Science, vol. 129, 1905, pp. 751–754.

[f] Studies on the Bactericidal Action of Copper on Organisms in Water. American Journal of Medical Science, vol. 129, 1905, pp. 754–760.

[g] A Study of the Action of Colloidal Solutions of Copper upon Bacillus Typhosus, American Journal of Medical Science, vol. 129, 1905, pp. 760–769.

[h] Buls. 64 and 76, Bureau of Plant Industry, U. S. Dept. of Agriculture.

drinking water by exposing it to the action of clean metallic copper.[a] Perhaps this opinion should be qualified by adding that the action is more rapid if the water contains no free carbon dioxid, altho the following tables show only slight differences between the toxicity of metals because of the presence or absence of carbon dioxid.

TABLE XXVIII.—*Effect of carbon dioxid upon toxicity of metals to Bacillus coli.*[1]

Duration of exposure to action of metals.	Check.	Copper.	Iron.[2]	Zinc.	Lead.	Tin.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	3,700	1,100	3,300	3,050	3,700	3,500
4 hours	440	75	30	125	275	410
8 hours	305	3	3	30	120	190

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of water triple distilled from glass, to portions of which were added sterile blocks of the proper metals, each having approximately 2 sq. cm. surface area. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

[2] Iron impure and presumably more toxic than pure iron. (See Table No. XXII.)

TABLE XXIX.—*Toxicity of metals to Bacillus coli in the absence of carbon dioxid.*[1]

Duration of exposure to action of metals.	Check.	Copper.	Iron.[2]	Zinc.	Lead.	Tin.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	2,950	2,350	1,200	1,450	1,300	1,600
4 hours	965	10	10	65	40	770
8 hours	740	0	0	5	5	700

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of water triple distilled from glass, to portions of which were added sterile blocks of the proper metals, each having approximately 2 sq. cms. surface area. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

[2] Iron impure and presumably more toxic than pure iron. (See Table No. XXII.)

[a] There is a seeming discrepancy in this statement due to the fact that Mr. Earle B. Phelps, assistant hydrographer, U. S. Geological Survey, has carried on experiments on the storage of typhoid infected water in copper canteens, some results of which, with conclusions of a nature unfavorable to the use of metallic copper in practically sterilizing water, were issued in a press circular of the Geological Survey. A quotation from Mr. Phelps's paper paralleled with a quotation from Bureau of Plant Industry Bulletin No. 76 shows clearly that Mr. Phelps's work is a corroboration instead of a contradiction of the fact reported in Bulletin No. 76 that metallic copper has a high germicidal value. Mr. Phelps has stated: "The fact that organisms do survive the copper treatment even in small numbers [Per cent reduction usually over 99.999.—K. F. K.] seems, in the writer's view, to lessen considerably the value of the canteen as a safeguard against typhoid infection. A second point of interest is the fact that the efficiency of the canteen decreases as time goes on, probably owing to the accumulation on the surface of the copper of a film of basic carbonate or other insoluble copper compound." A paragraph from Bureau of Plant Industry Bulletin No. 76 reads as follows: "Complete sterilization is a standard to which even the best filters seldom attain, and under the most unfavorable conditions the reduction in the number of bacteria in water exposed to the action of metallic copper for twelve hours will be approximately as great as filtered water. The copper must be kept clean, not, as is popularly supposed, to protect the consumer from copper poisoning, but because it is possible for the metal to become so coated with foreign substances that there is no longer any contact of copper and water, and hence no antiseptic action."

To determine whether the peculiar variation in the germicidal power of solutions or metals is due to the use of ordinary laboratory glassware made of a rather soluble glass instead of carefully selected highly insoluble glass, a parallel series in good and poor glass was carried on. The following tables show the slight difference in results:

TABLE XXX.—*Effect of glass upon toxicity of metals to Bacillus coli.*[1]

Duration of exposure to action of metals.	Check.	Copper.	Iron.	Zinc.	Lead.	Tin.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	2,600	1,925	1,850	2,450	2,600	3,100
2 hours	2,650	775	2,100	715	2,800	2,300
6 hours	2,700	525	2,350	70	2,450	4,200
24 hours	28,000	15	4,350	1	13,750	12,000

[1] Experiment conducted in ordinary glass test tubes each containing 10 c. c. of water triple distilled from glass, to portions of which were added sterile blocks of the proper metals, each having approximately 2 sq. cm. surface area. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

TABLE XXXI.—*Effect of glass upon toxicity of metals to Bacillus coli.*[1]

Duration of exposure to action of metals.	Check.	Copper.	Iron.	Zinc.	Lead.	Tin.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	2,300	4,825	4,750	2,400	2,800	3,000
2 hours	1,572	975	2,400	200	1,800	3,600
6 hours	1,400	190	3,050	20	2,000	3,850
24 hours	10,825	1	6,700	1	19,000	14,000

[1] Experiment conducted in Weber resistance glass test tubes each containing 10 c. c. of water triple distilled from glass, to portions of which were added sterile blocks of the proper metals, each having approximately 2 sq. cm. surface area. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

COPPER SULFATE AND FILTRATION.

The use of copper sulfate in connection with filtration has been mentioned in previous bulletins. Further experiments in this field show that in mechanical filtration with alum it is necessary to limit the use of copper sulfate to treatment some hours before coagulation. When solutions of aluminum sulfate and copper sulfate are mixt and alkali or hard water is added in quantities sufficient to cause precipitation the copper is coagulated at once, while the aluminum is deposited on the copper and incloses it, with the result that the copper-alum coagulum is no more toxic than is the pure alum coagulum. When copper and iron salts are precipitated together the reverse of this seems to take place and the precipitate retains its toxic properties.[a]

[a] See also H. W. Clark, Sulphate of Alumina as a Germicide. Thirty-sixth Annual Report of State Board of Health of Massachusetts, 1904, p. 288.

The following table shows the various combinations of precipitates tested and the exact results:

TABLE XXXII.—*Toxicity of combined precipitates to Bacillus coli.*[1]

Duration of exposure to action of precipitate.	Check.	Copper hydrate.	Iron hydrate.	Aluminum hydrate.	Copper and iron hydrate.	Copper and aluminum hydrate.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	2,250	1,525	645	7,750	3,450	1,750
2 hours	675	0	230	1,800	25	320
6 hours	465	0	230	195	0	15
24 hours	165	0	230	5	0	1

[1] Experiment conducted in 100 c.c. Jena glass flasks, each containing 15 mg. of the proper precipitate. All flasks inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

TABLE XXXIII.—*Toxicity of combined precipitates to Bacillus coli.*[1]

Duration of exposure to action of precipitate.	Check.	Copper hydrate.	Iron hydrate.	Aluminum hydrate.	Copper and iron hydrate.	Copper and aluminum hydrate.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	3,350	380	1,065	2,650	3,100	2,350
2 hours	305	0	1,900	3	1	80
6 hours	350	1	1,350	1	0	0
24 hours	200	0	850	1	0	0

[1] Experiment conducted in 100 c.c. Jena glass flasks, each containing 15 mg. of the proper precipitate. All flasks inoculated with a 2 mm. loop of culture of *Bacillus coli* received from the Bureau of Animal Industry, isolated from hog. The temperature during this experiment varied from 18° to 22° C.

The presence or absence of carbon dioxid is probably important in this connection. If the laboratory results will hold for field conditions, a copper precipitate or a precipitate of iron and copper will be highly toxic to *Bacillus coli* and *Bacillus typhi* in a water whose alkalinity is chiefly monocarbonate; and, conversely, the action of a copper precipitate or a copper-iron precipitate will be reduced if a water contains free carbon dioxid. This is probably the reason that small quantities of copper are toxic[a] in a mechanical filter using the proper quantities of iron and copper, and gives an additional reason for the advice given by Ellms[b] and Brown[c] that before filtration or distribution of a copper-treated water all free carbon dioxid and part of the semicombined carbon dioxid should be neutralized by caustic lime. Copper treatment of water previous to slow sand filtration should be made under similar conditions, and as this is seldom practicable it is perhaps advisable to limit the use of copper in connection with slow sand filtration to treatment after passing the filter, and before distribution the proper quantity of caustic lime may be added. An excel-

[a] Bul. 76, Bureau of Plant Industry, U. S. Dept. of Agriculture.

[b] Journal New England Water Works Association, vol. 19, 1905, pp. 496–503.

[c] Journal New England Water Works Association, vol. 19, 1905, p. 578.

lent example of the results to be expected from incorrect use of copper in connection with slow sand filtration is furnished in Clark's[a] report. Not only was copper found in the effluent of the experimental filter, but *Bacillus coli* was found in a rather high per cent of the bacterial samples.

The carbon dioxid content of the Ohio River is a possible explanation also of the fact reported by Brown[b] that samples of Ohio River water yielded between 0.00772 and 0.00657 grain of metallic copper to the gallon, tho *Bacillus coli* was occasionally present. To use his own words, "It is seen from the work done, as above stated, that the copper is present in an insoluble form and probably as the oxide united with the suspended mineral matter which the water carries. If in this form it would be unable to exert any germicidal power, and, as far as could be determined, this seems to be the case. It was decided that the copper was present in a spent condition." As there was no monocarbonate alkalinity noted, the Ohio River water at this time doubtless contained some free carbon dioxid, and from the analogy of the laboratory results it seems fair to assume that Brown's explanation must be supplemented by the theory of the heightened resistance of *Bacillus coli* due to carbon dioxid.

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[a] Journal New England Water Works Association, vol. 19, 1905, pp. 503–505.

[b] The Purification of Water at Marietta, Ohio. Official Report to Board of Public Service, 1906.

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